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USE OF COPPER SOAP DUST AS A FUNGICIDE

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INTRODUCTION

Copper soap dust, a new fungicide made from copper sulphate and either resin fish-oil soap or caustic potash fish-oil soap, has in experiments conducted for two years at the Arlington experimental farm, Rosslyn, Va., given as good control of tomato leaf spot (*Septoria lycopersici*) as either 4-4-50 liquid Bordeaux mixture or Sanders' Bordeaux dust.

As copper soap dust seems to possess very desirable properties for the control of nearly all foliage diseases the results of its effect on the control of tomato leaf spot, although limited to two seasons' use, and its method of preparation are described for the benefit of those engaged in dusting and spraying experiments on other crops.

Copper soap is similar in composition to ordinary potassium or sodium soaps used for washing purposes but contains copper instead of potassium or sodium. Like other soaps its chemical composition is determined by the composition of the oils or fats used in its preparation. As the copper soap used by the writers was made from fish-oil soaps it probably consisted of a mixture of copper stearate, copper palmitate, copper oleate, and other similar copper salts of fatty acids.

PREPARATION OF MATERIAL

This new fungicide was made from concentrated, rather than dilute, solutions of copper sulphate and soap, because of the greater convenience and economy in handling the precipitate. A hot aqueous solution of resin fish-oil soap or potash fish-oil soap solution of about the consistency of syrup was poured into a saturated solution of copper sulphate and stirred to bring all the soap into contact with the copper. The soap solution was made just thin enough to cause a chemical reaction with the copper sulphate. The precipitate formed by the concentrated so-

lutions was as thick as mush. To prevent copper sulphate and soap from being thrown out of such concentrated solutions, the mixture was kept moderately hot until the reaction was completed. The potassium sulphate formed during the chemical readjustment was left in the mixture but whether or not it is of any importance was not determined by experiment. The precipitate was dried in the air and ground to a powder which varied in fineness owing to the difficulty of grinding copper soap without the aid of special machinery.

Copper soap dusts containing crystalline copper sulphate and soap in proportions of 1-4 and 1-6, respectively, by weight were prepared from both resin fish-oil soap[1] and caustic potash fish-oil soap.[1]

Lead arsenate was used at the rate of 2 pounds to 1 pound of crystalline copper sulphate with certain portions of these dusts in 1919 and calcium arsenate at the rate of ½ pound to 1 pound of crystalline copper sulphate in 1920.

METHOD OF DUSTING AND SPRAYING.

The dusting was done by means of a small hand duster. In 1919 a wire screen cylinder covered with cheesecloth was set over each plant during the dusting to prevent the dust from spreading to neighboring plants. In 1920 the dusts were applied during periods of still weather without the use of the screen. As plats instead of rows were used in 1920 and the two outside rows of each plat were discarded, it was possible by careful use of the hand duster to prevent the dusts from spreading to the experimental rows of neighboring plats.

The liquid sprays used for comparison with the dusts were applied by means of a compressed air sprayer.

RESULTS OF EXPERIMENTS IN 1919

Copper soap dusts 1-4 and 1-6 and copper soap lead arsenate dusts 1-4-2 and 1-6-2 prepared from both resin fish-oil soap and potash fish-oil soap were used in the dusting experiments in 1919. Liquid copper soap sprays and Bordeaux mixture were also used for comparison. Single rows 64 feet long were used as plats. Several replications of each treatment were made in different parts of the field. As every third row was left untreated the dusted rows will be compared with both the sprayed and the untreated rows.

The dusts were applied at the rate of 13.2 pounds per acre and the liquid sprays at the rate of 100 gallons per acre at each application.

[1] These were commercial soaps. The resin fish-oil soap contained about 48 per cent resin and 16 to 20 per cent water; the caustic potash fish-oil soap, about 25 to 30 per cent water.

As the 1-4 and 1-6 dusts made from resin fish-oil soap and from potash fish-oil soap seemed to be equally effective in the control of tomato leaf-spot, the results from these two treatments will be combined in one group and the results of all rows treated with a mixture of either of these dusts and lead arsenate will be summarized in another group. Summaries of the results obtained from these groups and from the use of liquid copper soap and of liquid Bordeaux mixture are presented in table 1.

TABLE 1

Comparison of copper soap dust, copper soap liquid, and Bordeaux mixture in the control of tomato leaf spot (Septoria lycopersici) at Arlington Experimental Farm, 1919

Dust or spray liquid	Period of treatment		Increased yield from dusting or spraying	No. of applications	No. of separate treated rows	No. of separate check rows
	Early	Late				
Copper soap dust....	.....	7/25–9/19	16.7%	3	8	16
Copper soap dust and lead arsenate..	.....	7/25–9/19	31.1%	3	6	12
Bordeaux mixture 4–4–50.....	.....	7/25–9/18	11.3%	4	2	4
Copper soap liquid*..	.....	7/25–9/18	12.8%	4	6	12
Copper soap liquid and lead arsenate..	.....	7/25–9/18	36.5%	4	6	12
Bordeaux mixture....	6/21–9/4		—5.4%	5	2	4
Copper soap dust....	6/20–9/5		1.1%	4	8	16
Copper soap dust and lead arsenate..	6/20–9/5		15.7%	4	7	14
Copper soap liquid..	6/25–9/5		—8.6%	5	6	12
Copper soap liquid and l ad arsenate..	6/25–9/5		—4.9%	5	6	12

Late dusting with copper soap dust gave fully as large increases in yield of fruit as late spraying with either liquid Bordeaux mixture or liquid copper soap. The increases would probably have been greater if leaf spot had appeared earlier.

Although the rows receiving the last treatment on September 5, received a larger number of applications of fungicide than those treated until September 19, they produced less fruit, as leaf spot appeared late

* The copper soap sprays contained ½ pound of copper sulphate and 3 pounds of resin fish-oil soap in 50 gallons of liquid or one-eighth as much copper as the 4–4–50 Bordeaux mixture. Full directions for preparing copper soap liquid may be obtained from Phytopathology **9**: 554–564. 1919.

and developed chiefly in the latter part of the growing season when they were not so well protected from its attack.

The apparent reductions in yield obtained from early spraying with Bordeaux mixture and with liquid copper soap may be due either to variations in soil fertility of the sprayed and check plats or to injury from the fungicides. Similar results from early spraying have been observed in other experiments.

The large increases in yield of fruit from the use of a combination of lead arsenate and the fungicides over the fungicides alone are difficult to explain. They may have been due to insect control but no insect infestation was apparent. Moreover, similar benefits from the addition of lead arsenate to copper soap liquids were obtained in control of tomato leaf spot on the foliage in former greenhouse experiments although no benefits were obtained from lead arsenate alone.

RESULTS OF EXPERIMENTS IN 1920.

In the dusting experiments in 1920 copper soap calcium arsenate dust of the 1-6-½ formula and Sander's Bordeaux dust of the 15-8-77 formula, i. e., 15 pounds of dehydrated copper sulphate, 8 pounds of calcium arsenate, and 77 pounds of dehydrated lime, were used at the rate of 22½ and 30 pounds per acre, respectively, at each application. Bordeaux mixture of the 4-4-50 formula was also used at the rate of 100 gallons per acre for comparative purposes.

Four parallel 5-row plats of tomatoes 230 feet long were used in these experiments, one for each of the dusts, one for the Bordeaux mixture, and one for control purposes. The control or check plat lay between the Sanders' Bordeaux and copper soap calcium arsenate plats. Data from each plat were taken only from the three central rows.

Five applications of the fungicides were made between August 3 and September 15. But little if any effect on yield of fruit was expected, as the crop was nearly grown when the experiment was started. The treatments were continued to September 15 to observe their effect on the preservation of the foliage.

Tomato leaf spot (*Septoria lycopersici*) and tomato leaf mold (*Cladosporium fulvum*) were both doing considerable damage when the spraying was begun. Although leaf spot was checked by the fungicides, leaf mold continued to cause considerable defoliation. The Cladosporium spores lying on the lower leaf surfaces beyond the effective reach of the chemicals germinated through aid of unusually moist weather and infected the newly developed foliage before it was covered with fungicides.

The increased yields from the treatments, though of little importance, for reasons already stated are expressed in percentages as follows: San-

ders' Bordeaux dust—13.7, copper soap calcium arsenate dust 1. 5 and liquid Brodeaux mixture 5.4. It would seem from these results that Sanders' Bordeaux dust caused some injury, though an allowance of the greater part of this decrease would have to be made for experimental error. The small increases from the copper soap calcium arsenate and Bordeaux plats are also doubtfully significant, as they do not exceed average differences due to variations in soil fertility. In fact, little if any increase could have been expected as one of the heaviest pickings of fruit was made on the day the experiment started and nearly all the crop was harvested by September 1. Based on preservation of the foliage, however, Bordeaux mixture seemed to rank first, with copper soap calcium arsenate dust and Sanders' Bordeaux dust, a close second and third. As Bordeaux mixture is one of the most effective spray substances ever used and as Sanders' Bordeaux dust is being successfully substituted for it in Nova Scotia, Canada, and in some parts of the United States, the showing made by copper soap dust in comparison with these fungicides looks very promising.

DISCUSSION

There is much need of an economical dust fungicide that will give as effective results as liquid Bordeaux mixture. Although many dusts have been tried, none with the possible exception of Sanders' Bordeaux, has given as good results for general use as liquid Bordeaux.

Copper soap dust seems promising but its merits can not be definitely determined until it is thoroughly tested on a large number of foliage diseases. It has some advantages over Sanders' Bordeaux, as it spreads more evenly and floats and adheres better. Moreover, it is free from a lot of inert material commonly present in most dust fungicides, and therefore distributes the copper uniformly. Because of its light weight and floating quality, it also has a better opportunity to come into contact with the foliage than has Sanders' Bordeaux or other heavy dusts.

The copper soap dust experimented with gave good results in spite of the fact that its particles varied greatly in size. If ground uniformly fine it would not only have greater covering capacity but should give better results.

Copper soap dust will probably cost about as much as Sanders' Bordeaux if, as these experiments seem to indicate, 15 pounds of copper soap dust are as effective as 30 pounds of Sanders' Bordeaux. As 13.2 pounds of copper soap dust gave as good control of Septoria leaf spot in 1919 as 100 gallons of 4-4-50 liquid Bordeaux mixture, 15 or 16 pounds per acre may suffice for the control of tomato foliage diseases. If on this estimate we compare the cost of dusting with copper soap and lead arsenate with

that of spraying with 4-4-50 liquid Bordeaux mixture and lead arsenate, even at the rate of 50 gallons of mixture per acre instead of 100 gallons as used in the experiments, the relative costs per acre are given in table 2.

TABLE 2

4–4–2–50 Bordeaux lead arsenate mixture

50 gallons per acre	1 application	5 applications
4 lbs. copper sulphate at 10c	$0.40	$2.00
4 lbs. lime at ½c	.02	.10
2 lbs. lead arsenate at 40c	.80	4.00
* applications of 50 gals. at 2c	1.00	5.00
	$2.22	$11.10

1–6–1 copper soap lead arsenate dust

16 lbs. per acre	1 application	5 applications
2 lbs. copper sulphate at 10c	$0.20	$1.00
12 lbs. resin fish-oil soap at 6c	.72	3.60
2 lbs. lead arsenate at 40c	.80	4.00
* application 16 lbs. at ½c	.08	.40
	$1.80	$9.00

By dispensing with the insecticide the expense of using either fungicide would be reduced 80 cents for each application or $4.00 for five applications.

The estimated cost of using copper soap dust is less than for the use of liquid Bordeaux mixture but no allowance has been made for grinding and mixing. On the other hand, the cost of Bordeaux has been calculated on the basis of 50 gallons an acre, while it was used in the experiments at the rate of 100 gallons an acre. Moreover, the writers have found that 50 gallons an acre are not so effective in the control of tomato leaf spot (*Septoria lycopersici*) as 100 gallons.

Copper soap dust of the 1-6 formula should be tested both with and without the addition of lead arsenate for the control of foliage diseases of a large number of crops. Although the other formulas and mixtures should also be tried, they should not be used on a commercial scale until their properties are better known.

SUMMARY

Copper soap dust, a new fungicide prepared from copper sulphate and fish-oil soap, has for two years at the the Arlington experimental farm

* The estimated cost of application by Sanders and Kelsall (Proc. Entomol. Soc. of Nova Scotia, 1918, p. 32) is 2 cents a gallon for liquid and ½ cent a pound for dusts, but this is probably low for spraying and dusting field crops. However, if a 6-row duster were used the cost of dusting would be very little per acre.

given as good control of tomato leaf spot (*Septoria lycopersici*) as 4-4-50 liquid Bordeaux mixture. It spreads, floats, and adheres better than Sanders' Bordeaux dust and in these experiments gave better control of tomato leaf spot. As it is much more effective pound for pound than Sanders' Bordeaux, it will probably cost no more per acre. Moreover, it is cheaper than liquid Bordeaux, considering the cost of application, and is much more easily and quickly applied. Because of its excellent chemical, physical, and fungicidal properties, it offers promising possibilities for the control of a large number of other foliage diseases.

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STUDIES ON THE VALSA APPLE CANKER IN NEW MEXICO

LEON H. LEONIAN

Some specimens of cankered apple branches were sent to the New Mexico Agricultural Experiment Station from Socorro county, N. M. in the winter of 1919. The farmer, who submitted them, claimed that a large number of his trees, both young and old were rapidly being killed by the disease. The following spring the same canker made its appearance on the apple trees of the Station farm, and furnished ample material and opportunity to study the disease. A preliminary survey showed that the canker was present in almost every apple growing section of New Mexico, and that it was believed by the growers to be very troublesome.

THE DISEASE

The canker appears on the twigs, the branches, and the main trunk of young or old apple trees. If the point of primary infection is on the twig, the canker spreads downward and into the branches and the trunk; but if it is on the trunk, it spreads upward, but more slowly, and before it reaches all the branches, the trunk may be completely girdled.

The disease shows itself first by forming discolored, sunken lesions which may extend along the entire length of the branches and eventually affect all sound tissues. The area between the diseased and the healthy wood is well marked by the discolored and sunken nature of the invaded region, by a wrinkled, peeling and split epidermal border, and often by the appearance of a purplish zone which usually marks the advance of the fungus. The infected bark is at first water-soaked, and the epidermis can readily be rubbed off exposing darkened tissues beneath. The fruiting bodies of the fungus appear over the entire cankered area, generally one or two weeks after the forming of the lesions. The symptoms on the trunk are somewhat different; the water-soaked bark becomes soft, swollen, and of cheesy consistency when pressed with the fingers. Gradually, however, it dries and sinks back to the level of the unaffected tissues. As the drying progresses, it shrivels still further, while the border between the cankered and the healthy parts cracks, and the loose epidermis gathers itself into semi-transparent wrinklings.

[1] Published by the permission of the Director of the New Mexico Agricultural Experiment Station. The writer wishes to express his thanks to Dr. C. H. Kauffman for his critical reading of the manuscript and helpful suggestions.

If the canker is on the trunk, the tree gradually turns sickly looking, the leaves remain small, fade into a faint green, then become yellowish and drop off. The fruit remains undersized, juiceless, and soon falls. Neither a sudden, nor even a gradual wilting has been observed. Somewhat different effects result when the canker starts on the branches; the affected tissues dry more rapidly, and defoliation takes place much faster. The canker extends from branch to branch, but its rate of advance depends entirely upon the condition of the tree, so that often the final destruction may not be accomplished before two or even more years.

THE FUNGUS

The fruiting bodies of the fungus responsible for the canker are largely pycnidia, with well developed, convex, multipycnidial stromata, and hyaline, non-septate, allantoid conidia. In this stage it is, therefore, a species of Cytospora. To refer this fungus to the proper species presented a difficult problem because of the very large number of closely resembling forms classified under this genus. At first a careful search for the perfect stage of the fungus met with negative results. Meanwhile Stevens (6) published a bulletin in which he discussed a similar canker of the apple caused by a species of Cytospora. Some samples of cankered specimens and pure cultures of the causal organism were sent to Dr. Stevens for comparison, and he declared the organisms to be identical. Finally the writer's search for the perfect stage of the fungus was rewarded by discovering a small patch of fruiting bodies which proved to be the perithecia of a Valsa species resembling *Valsa leucostoma* (Pers.) Fr. as described so extensively by Aderhold (1) and Rolfs (3, 4, 5).

IN CULTURE

The relationship of the perfect to the imperfect form of this fungus was established by pure cultures and inoculation experiments. Ascospore isolations upon steamed apple twigs gave rise to both the Cytospora and the Valsa type of fruiting bodies. When conidia thus obtained were transferred to another series of steamed apple twigs, they yielded nothing but pycnidial stromata, while similar cultures with ascospores produced both pycnidia and perithecia. Inoculation experiments in the field with the conidia and the ascospores of this fungus, as well as with conidia obtained from pure cultures of a plum strain of *Cytospora leucostoma*, gave rise to lesions and fruiting bodies identical with those found on the cankered apple branches in the orchard. Pure cultures of *Cytospora chrysosperma* (Pers.) Fr. were used as checks and were found to be unable to affect the apple trees. When grown on apple branches, the plum strain of this fungus lost the characteristic white substance which

ordinarily crowns its ostioles, and its stromata no longer adhered to the epidermis, but became more closely imbedded in the bark. In the New Mexico climate the apple strain of this fungus does not show any white, powdery substance on the ostioles; but in test tube cultures, where the moisture conditions have been controlled carefully, it appears. This white substance is nothing more than masses of stromatic hyphae which being exposed to the air, dry and become powdery. The epidermis of the affected apple branches either does not rupture under the pressure of the enlarging stromata beneath, or else it merely cracks, without, however, opening wide enough, as is the case with that of the plum trees, to expose the gray stromatic masses. Probably the epidermis of the apple branches is more elastic and tenacious. In test tubes, however, where moisture conditions are properly controlled, the stromatic tissues expand more forcefully and overcome the resistance of the epidermis, pushing out the gray masses through the cracking and yielding host tissues.

The mycelium of this fungus grows well and pycnidia form readily on oatmeal and cornmeal agars, on potato hard agar containing five per cent glucose, and on steamed apple twigs and sweet clover stems. Very sparse mycelial growth and no pycnidial development was observed on nutrient gelatin (+1). On nutrient agar[2] the mycelium was mostly submerged, moist, and very poor. Some dark, olivaceous hyphae formed compact, wrinkled crusts very similar to pycnidial walls, but no spores were ever found in these. When the quantity of agar-agar was raised to seven per cent or more, however, and even on pure agar-agar, a few undersized stromata containing mature pycnidia developed. Coon's (6) synthetic solution[3] made solid by adding 1. 5 per cent agar-agar, was found to induce the formation of a very few mature pycnidial fruiting bodies. The mycelium made a poor growth on this agar, however, and mature pycnidia were not observed until fifteen days after the time of inoculation, whereas on cornmeal and oatmeal agars the first fruiting bodies appeared within four days, and a week later the entire slant of the agar became thickly warted with well developed fruiting bodies. On

[2] Ammonium nitrate 1 gm., dihydrogen potassium phosphate .5 gm., magnesium sulphate .25 gm., iron chloride trace, cane sugar 5 gms., agar-agar 1.5 gm., water 100 c.c.

[3] "Magnesium sulphate + 7 aq. 2 46 gms., in 50 c.c. water; potassium acid phosphate 1.36 gms. in 50 c.c. water; asparagin 1.33 gm. in 50 c c water; maltose 3.60 gms., in 50 c.c. water. For 100 c.c. synthetic solution take 1 c.c. magnesium sulphate, 5 c.c. of each of the other solutions and add to 84 c.c. water. Steam in Arnold's sterilizer for three successive days." Wherever in this paper reference is made to Coon's media, it should be understood that the solution was made solid by the addition of 1.5 per cent. agar-agar.

steamed apple twigs which did not contain a very large amount of moisture, the stromata developed normally, and except on the cut ends of the twigs, no hyphal growth could be seen. When the moisture content was too high, however, and the air in the test tubes was saturated with water vapor, the stromata became entirely superficial, strongly globose, often verruciform, and coated with a gray to olive green hyphal velvet. On artificial media and in a very moist atmosphere the pycnidial stromata became stalked. At first a number of hyphal wefts appear; these form slender pillars two to three mm. tall. The upper parts of these branch out into cottony, loose heads in the form of bouquets. Later, in the central portion of these heads there appear some olivaceous hyphae which become compact, round up and form mature stromata. Often more than one stroma may appear, supported by the same column.

Numerous attempts to obtain perithecia on oatmeal agar failed until the composition of the media was changed by the addition of various amounts of sugar. Three weeks after inoculation with ascospores the entire slant of the agar containing sugar became bristling with the beaks of the perithecia. The number of perithecia increased and that of pycnidia with the gradual augmentation of the sugar content. The period for the formation of perithecia was lengthened, however, with the higher concentrations of sugar, so that while on media with 2 per cent cane sugar the perithecia appeared within 10 to 12 days, on those with 8 to 12 per cent of sugar they were delayed for more than a month. Different results were obtained on cornmeal agar; perithecia formed readily on this when it contained no sugar, while an addition of more than 2 per cent sugar delayed, or even inhibited their formation. Sodium chloride added to oatmeal agar acted the same way as cane sugar, although in this instance the period of perithecial development was lengthened to five weeks. Enough sodium chloride and cane sugar were weighed to make M/16 solutions of each, and added to two separate lots of oatmeal agar. In case of oatmeal agar containing sugar a great abundance of perithecia was observed in about three weeks, while in case of oatmeal agar containing sodium chloride, five weeks after an equally large abundance of perithecia was obtained. The checks, to which neither sugar nor salt were added, produced pycnidia only, although after three months desiccation in the laboratory, about half a dozen perithecia formed on the driest edge of this media.

Interesting results were obtained when cultures of this fungus were transferred from one solution to another. A new cultural method was devised to conduct these experiments. The basic principle of this was derived from Coon's (2) filter paper cone system. He grew his fungus on filter paper cones kept in glass capsules which contained some nutrient

solution. While this method makes it possible to transfer the entire growth of a given fungus from one solution to another without disturbing the mycelium, it confines the investigator to liquid cultures, failing, at the same time, to eliminate the disadvantages of the old methods,namely the evaporation of the water from the media and the consequent increase of its osmotic pressure. The following variation of the cone method has therefore been used; filter paper cones of any desired size are made, the apex of the cone is moistened and pushed in with the finger to give it a cup-like appearance, then glass capsules of suitable size are selected and filled two-thirds with distilled water, one cup being placed in each and sterilized. Any solid media can then be poured into the cups. Care should be taken to pour just before it begins to jell, else it may filter through the cone. With this treatment the moisture content of the media was observed to remain practically the same during the expreiment. This was due to the fact that water from the capsule passsed up into the media by capillarity and replaced the evaporating moisture.

When the cultures, in Coon's synthetic media (3) were repeated in the laboratory at Ann Arbor, it was found that the pycnidia were slower to appear. They developed at least two weeks later than under similar cultural conditions in the New Mexico laboratory. It was found that their development could be hastened considerably by adding cane sugar or sodium chloride to the media, as follows: the synthetic media was poured in filter paper cups which were kept in glass capsules containing distilled water. Six days after inoculation the cultures were divided into three lots; one served as check, while the cups of the second lot were transferred, by means of flamed forceps, to other sterile capsules containing M/16 sodium chloride solution, and those of the third lot were transferred to capsules with M/16 cane sugar solution. Ten days later a fair number of pycnidia formed on the media of the second and the third lot, while in case of the checks no pycnidia developed even after one month.

TABLE 1

Culture cups used with solutions

Set 1.	Check.	Culture cups allowed to remain in distilled water.
Set 2.	Check.	Culture cups allowed to remain in M/16 cane sugar solution.
Set 3.	Check.	Culture cups allowed to remain in M/16 sodium chloride solution.
Set 4.		Cultures transferred from distilled water to M/16 cane sugar solution.
Set 5.		Cultures transferred from distilled water to M/16 sodium chloride solution.
Set 6.		Cultures transferred from M/16 sugar solution to distilled water.
Set 7.		Cultures transferred from M/16 sodium chloride solution to distilled water.

Since the synthetic media failed to induce the formation of the asci-

gerous stage, and since oatmeal agar reacted so well when it contained either salt or sugar throughout the growth of the fungus, it was believed that when subjected to the action of sodium chloride or sugar temporarily, it would produce interesting variations in the fungus. With this end in view the following experiments were made: a series of filter paper cup cultures were made and the cups were filled with plain oatmeal agar. Some of the capsules contained distilled water, others M / 16 cane sugar solution, and a third lot M / 16 sodium chloride solution. Thus in one case the oatmeal agar received distilled water through the capillary action of the filter paper, in the second case it imbibed sugar, and in the third sodium chloride. All of these cultures were inoculated on the same date. Six days after, when the fungus had made a fair growth but no fruiting bodies had appeared as yet, the cultures were transferred from one solution to another as described in the foregoing table. All sets were in duplicate, and the experiments with the sugar were repeated. The cultures were washed in distilled sterile water before transferring from salt or sugar solutions to water.

Four days after the transfer, or ten days from the time of inoculation, pycnidia developed in all of the seven groups. It seems that the necessary stimulus for their formation was present in the oatmeal itself, and the addition of salt or sugar could have no marked effect. Entirely different results, however, were obtained in case of perithecia formation, as can be seen in the following table;

TABLE 2

Effect of above treatment of cultures on formation of perithecia

Set 1.	No perithecia, even after 36 days.
Set 2.	Perithecia formed in 29 days.
Set 3.	No perithecia, even after 36 days.
Set 4.	No perithecia, even after 36 days.
Set 5.	No perithecia, even after 36 days.
Set 6	Perithecia formed in 18 days.
Set 7	Perithecia formed in 29 days.

It can be seen, from the foregoing table, that the necessary stimulus for the formation of the perfect stage will have to be furnished from the very beginning of a period limited to 36 days, and that the absence of the stimulus for the first six days will be sufficient to suspend perithecia formation, even though it is furnished at the end of that period. The constant presence of sugar or sodium chloride retards the formation of the ascigerous stage for a considerable period. It will also be noted that in filter paper cup cultures perithecia require a longer period to appear than

they do in test tubes. Perhaps the gradual drying of the media in the test tubes is responsible for this effect.

PATHOGENICITY

To the casual observer this fungus will appear to be a parasite of great physiological efficiency. According to Stevens (6) "it appears that this canker is one of very rapid development and one that might cause serious loss should it become widespread." He made inoculations on apple and other twigs in test tubes with a few centimeters of water to keep the cultures moist and alive, and he obtained a ready growth and fruiting of his fungus. He did not care to make inoculations in the field for fear of spreading the disease. The writer selected a number of apple trees in the Station orchard, and inoculated them with pure cultures of this organism. Some of the trees were vigorous, others weak and gradually dying. Twigs, branches and trunks were inoculated with either conidia or ascospores. The inoculations were made through wounds in case of some trees, while no wound was made in case of others. None of the healthy trees showed any sign of infection even four months after the inoculation. In case of the weaker trees, however, the wound inoculations developed the canker within ten days, while none of the inoculations made on the sound tissues and given all the favorable conditions for infection, gave any positive result. Within a period of six weeks the fungus progressed from the tips of the twigs into the branches, the limbs, and the trunks, forming an abundance of typical fruiting bodies as it advanced. The rate of the progress of the canker was always governed by the condition of the tree; it was rapid if the tree was weak, and slow if it was less severely affected.

All trees which showed canker, either as a result of natural infection or of artificial inoculation, were dug out to determine the cause of their weakened condition. It was found that in all cases the roots were badly infected with the wooly apple aphis, or the underground trunk was almost completely girdled by the giant apple tree borer. Often both of these injuries were found to be associated. The organism which causes the canker, therefore, so far as conditions in New Mexico are concerned, is not a parasite to be feared, at least not for the time being. The same thing is true in case of plum and peach trees, which, if well cared for, never show any canker caused by *Cytospora leucostoma*. Old and neglected trees are the only ones which can be affected by this organism.

SUMMARY

A great number of apple trees throughout New Mexico were found to be badly affected with a canker caused by *Valsa leucostoma* (Pers.) Fr.

Twigs, branches, limbs, trunks may be attacked by the fungus with equal ease. Both the imperfect and the perfect stages of the organism responsible for the canker were reproduced in artificial media. Sodium chloride and cane sugar, under proper conditions, acted as stimuli to form perithecia on oatmeal agar.

Inoculation experiments showed the fungus to be a weak wound parasite.

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TOLERANCE TO ACIDS OF CERTAIN BACTERIAL PLANT PATHOGENES.

FREDERICK A. WOLF AND I. V. SHUNK

INTRODUCTION.

Cultural studies of plant pathogenic bacteria are generally believed to be incomplete unless they include a determination of the lethal or limiting hydrogen and hydroxyl ion concentration. Recent investigations on forms pathogenic to man have demonstrated, by the employment of refined methods, the importance and value of hydrogen ion determinations. Similar studies by plant pathologists, employing methods of precision in measuring acidity and alkalinity, in place of the meaningless titrimetric methods, would no doubt show that H-ion concentration is of significance in the culture of plant pathogenic forms. Certainly in such an important problem as the possible correlation between tolerance to acid and immunity or resistance no progress has been made with methods in vogue and no convincing body of evidence has thus far been adduced. Neither will such data be forthcoming until a better understanding is had of some of the fundamental factors underlying the inhibitory or antiseptic action of acids.

Further, the influence of the hydrogen ion inhibition of growth and on the processes of metabolism in bacteria is generally believed both to be specific and an easily explicable matter. It becomes obvious, however, that such is not the case, and that much additional experimentation is necessary before an adequate explanation can be offered for the phenomena which may be noted if one cultivates several species of bacteria on media which differ only in the kind of acids employed in making the pH adjustment.

In the present investigation, attention has been directed to the n ature of the acid and to the nature of the medium as factors in the problem of the influence of pH. There has arisen, outside the field of plant pathogenic bacteriology, a considerable body of literature dealing with inhibition and antiseptic action. Some of these investigations, as will be discussed later, point out the interesting fact of the failure of acids of the same pH concentration to inhibit cell multiplication, the cause of which has not been satisfactorily explained. This observation has been confirmed in the present study with plant pathogenic forms and in addition, differences in growth in liquid and solid media are noted which further indicate the complexity of the problem of inhibition.

METHODS

The organisms employed were *Bacillus carotovorous, Bacterium campestre, Bacterium angulatum, Bacterium tabacum, Bacterium glycineum* and *Bacterium sojae.* They were cultivated in bouillon consisting of 1 per cent Armour's peptone, 0.3 per cent Liebig's beef extract and 0.5 per cent NaCl and in nutrient agar prepared by adding two per cent agar to the bouillon. Two per cent of agar and 1 per cent of peptone were arbitrarily employed after some preliminary experimentation upon the effect of variation of the agar content and of the peptone between 1 and 3 per cent. As is well known, both of these substances, except in the case of pure agar modify the "buffer" action and consequently the amount of acid necessary to secure the desired pH but within the range of 1 to 3 per cent, they appear to be without effect on inhibition of growth.

After dissolving the materials, the media were neutralized, flasked in 200 c. c. quantities and sterilized. As soon as they had cooled to about 60 ° C, commercially pure concentrated acid of the appropriate kind was added, using aseptic precautions, to obtain the desired pH values. The pH values were determined colorimetrically by comparison with buffer color standards. These buffer solutions were checked electrometrically and were found to be less than their assigned value by .03 to .06 pH. After the media had been adjusted to the desired H-ion concentration, they were poured into sterile test tubes. Such a procedure as demonstrated in a previous paper (5) is of especial value in the preparation of solid media since it removes the necessity of sterilization after adjustment of reaction and thus does not modify the acidity nor destroy the jellifying power. All tubed media were incubated for 48 hours before inoculation to determine their freedom from contamination.

RESULTS

Growth in bouillon, as shown in table 1, was determined by the ability of the several organisms to cloud the media. In the case of abundant clouding, the sign + is employed, no visible growth is indicated by — and a slight clouding by —+.

Manifestly the H-ion concentration alone does not determine the limit of tolerance of these organisms since one would then expect irrespective of the acid, cellular multiplication to be checked at the same point. The several organisms are seen to be more tolerant to malic than to any other of the acids and to be inhibited most by acetic. At a pH value of 4.6, when malic acid was employed *Bacterium tabacum* made a slight growth, whereas with acetic acid, the limit lies between 5.8 to 6.0. In the case

TABLE I

Tolerance of various organisms to acid in bouillon

Name of Organism	pH 4.6							pH 4.8							pH 5.0							pH 5.3							pH 5.5		pH 5.8	pH 6.0
	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Hydrochloric	Sulphuric	Acetic	Citric	Tartaric	Malic	Formic	Hydrochloric	Sulph huric	Acetic	Citric	Tartaric	Malic	Formic	Acetic	Formic	Acetic	Acetic
Bacterium campestre	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacillus carotovorus	−	−	−	−	−	−	−	−	+	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Bacterium glycineum	−	−	−	−	−	−	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Bacterium sojae . . .	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Bacterium angulatum	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Bacterium tabacum.	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+

TABLE II

Tolerance of various organisms to acids in agar

Name of Organism		pH 4.4	pH 4.6	pH 4.8	pH 5.0	pH 5.4	pH 5.5	pH 5.6	pH 5.8
Bacterium campestre	Hydrochloric	−	−	+	+	−	−	−	+
	Sulphuric	+	−	+	+	−	−	−	+
	Oxalic	−	−	−	+	−	−	−	+
Bacillus carotovorus	Hydrochloric	−	−	+	+	−	−	−	+
	Sulphuric	−	+	+	+	−	−	−	+
	Oxalic	−	−	−	+	−	−	−	+
Bacterium glycineum	Hydrochloric	−	−	+	+	−	−	−	+
	Sulphuric	−	+	+	+	−	−	−	+
	Oxalic	−	−	−	+	−	−	−	+
Bacterium sojae	Hydrochloric	−	−	+	+	−	−	−	+
	Sulphuric	−	+	+	+	−	−	−	+
	Oxalic	−	−	−	+	−	−	−	+
Bacterium angulatum	Hydrochloric	+	+	+	+	−	−	−	+
	Sulphuric	+	+	+	+	−	−	−	+
	Oxalic	−	−	−	+	−	−	−	+
	Acetic	−	−	−	−	−	−	−	+
Bacterium tabacum	Hydrochloric	+	+	+	+	−	−	−	+
	Sulphuric	+	+	+	+	−	−	−	+
	Oxalic	+	+	+	+	−	−	−	+
	Acetic	−	−	−	+	−	−	−	+
	Acetic	−	−	−	+	−	−	−	+
	Acetic	+	−	−	+	−	−	−	+
	Acetic	+	+	+	+	+	+	+	+

of *Bacillus carotovorus*, inhibition by these two acids occupies a much more restricted range since growth was checked between 4.6 and 4.8 by malic acid and 5.3 is the corresponding limit with acetic acid. *Bacterium campestre* is considerably more intolerant to formic acid than is *Bacillus carotovorus* since 5.5 and 4.8 represent the respective limits.

Differences in vigor of growth on the agar slants are indicated by the same signs and the data are summarized in table 2.

The most striking fact exhibited by growth on solid media as contrasted with liquid media is the ability of the several organisms to withstand a greater H-ion concentration when grown on the former. In general, this difference appears to vary from 0.2 to 0.4 pH. Just as in liquid media, all organisms are less tolerant to acetic than to the other acids, *Bacillus carotovorus* appears to be considerably less sensitive to acetic acid, however, than any of the other organisms since its limit lies between 4.8 and 5.0 and the others between 5.5 and 5.8.

DISCUSSION

The fact that acids of different hydrogen ion concentration can exert a similar influence in checking bacterial growth has previously been noted, as has been indicated. Winslow and Lochridge (4) using *Bacillus coli* and *B. typhosus*, found that the disinfecting power of acids was proportional to the concentration of the hydrogen ion, but noted in the case of acetic acid an effect greater than that due to the H-ion concentration. In explanation, they suggest that this is due to the undissociated acid remaining in the solution. More recently, Cohen and Clark (1) from cultural studies on *B. coli* pointed out that the factor or factors which obscure the effect of the H-ion are quite unknown. They noted that this organism grew well at pH 5.0 when adjustment was made with HCl but at pH 5.45 with acetic acid the same organism declined in numbers. Other organisms exhibited the same difference in conduct with reference to these two acids. They explain this as probably due to a synergic effect of the free acetate radicle upon the disinfecting power of the hydrogen ion, a view in accord with those who hold that the anions and undissociated molecules play an important role in the kinetics of disinfection by accelerating the action of the hydrogen ion. They also suggest the probability that this difference corresponds to a difference in permeability of the organism to mineral and organic acid.

Were one in possession of full knowledge of the unknown factor involved in the foregoing observations, he could no doubt explain what is believed to be a comparable condition reported by Svanberg (3). He found that *Streptococcus lactis* (*Bacterium lactis acidi*) in milk is capable of producing an acidity which corresponds to the value pH=4. When

however, lactic acid is added to milk, growth is checked at pH = 4.4 to 4.7 and acetic acid at pH = 4.8 to 5.1.

The plant pathogens used in the present investigation appear to respond differently toward the various acids just as did the several organisms employed in these several studies. This may be taken to indicate that the various acids exert a specific effect. Granted that the effect is specific, it is not clear how one could employ this fact in explaining the general shift of the limit of tolerance of 0.2 to 0.4 pH as occurs between bouillon and nutrient agar. It is felt, however, that this observation should be reported at this time on its own merit, subject to verification, and with the admission that no adequate explanation of the cause is known to us. It is believed that the fact of a greater pH tolerance on solid media is of significance in studies which attempt to correlate the acidity of juices of certain plants with tolerance in culture of forms parasitic on these same plants.

In conclusion, the present studies appear to be in accord with several others previously discussed in showing that the activity of the hydrogen ions alone is not responsible for the inhibitory effects which have been observed. This is opposed by the conclusions of a number of investigations, among which are those of Norton and Hsu (2) who inclined to the view that the disinfecting power of an acid is approximately proportional to the hydrogen ion concentration. Whatever may be the ultimate solution of this problem, it is apparent that further developments will depend upon the devising of methods which will show what are the dissociation products of the various acids in such complex solvents as bouillon or agar and whether or not these dissociation products in the presence of the H-ions modify the permeability of the bacterial cell wall.

SUMMARY.

Cultural studies have been made on the tolerance to acids of various plant pathogenic bacteria including the cabbage black rot organism, one commonly causing decay of root crops, two causing leaf spots of soy bean and two causing tobacco leaf spots.

Different acids of the same H-ion concentration are not able to exert the same influence in inhibiting cell multiplication. Acetic is more toxic than any other of the acids employed, an observation which accords with that of others.

A greater pH concentration in agar than in bouillon is required to inhibit growth, this difference ranging between 0.2 and 0.4 pH with the same organism and the same acid.

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PHYTOPATHOLOGICAL NOTES

Fiji disease of sugar cane in the Philippine Islands.—Fiji disease of sugar cane, previously reported only from the Fiji Islands, New Guinea, and Australia, has been found in the Philippine Islands. It was found on the Island of Mindoro simultaneously by Professor Reinking of the College of Agriculture, University of the Philippines, and the writer. Later the disease was found in Laguna and Batangas Provinces and was there identified by Mr. H. Atherton Lee, just returned from Honolulu where, through the kindness of Dr. H. L. Lyon of the Experiment Station, Hawaiian Sugar Planters' Association, he had the opportunity of examining preserved specimens of the disease from Fiji. There can be no question as to the identity of the disease.

The varieties Hawaii 109 and Java 247 are rather severely affected. Yellow Caledonia, Louisiana Striped and the native cane varieties Luzon White, Pampanga Red, and Cebu Purple were all affected, but at this time appear to have a slightly lesser degree of susceptibility.

In provinces where the disease is found, considerable losses have been caused on all of these varieties. One field of Yellow Caledonia plant was so severely affected that it was plowed under. In other fields, plantation managers estimate their losses at from 25 to 50 per cent, although the writer believes these losses possibly are a little over estimated. The losses from the disease are apparently increasing from year to year. The ratoon fields seem to show more of the disease than fields of plant cane.

To minimize or entirely prevent the losses from the disease, the following measures have been taken:

(1) The Plant Quarantine Board of the Philippine Islands has issued a regulation prohibiting the movement of all cane materials from Mindoro, Laguna, and Batangas Provinces. No case of the disease has been reported as yet in Negros, the large sugar-producing island; nor has it been reported from Cebu or Mindanao. In the absence of a definite report of the disease in those islands the domestic quarantine of the affected provinces seems justified.

(2) In cooperation with plantation managers in quarantined localities, steps are being taken to furnish seed points from disease-free fields, to planters whose fields are affected.

(3) Through the kindness of Dr. H. L. Lyon information has been made available concerning resistant varieties. Dr. Lyon states that Badila, Rose Bamboo, and Striped Singapore varieties, although not

immune, exhibit a degree of resistance to the disease. These varieties are available at the Plant Pathology laboratories and will be distributed in small amounts to planters in affected localities.—MARIANO G. MEDALLA.

The susceptibility of Dwarf Milo sorghum to smut.—The smuts of sorghum are of such an economic importance in India that in the Bombay Presidency alone, the annual losses caused by them exceed a million sterling. As most of the known varieties of sorghum are susceptible to smut the discovery of any immune variety will be a real boon to the cultivator. Attempts of this line of work have so far not been met with any success [1]. The writer's attention was drawn to the statement, occurring in the Journal of Agricultural Research.[2] "Fortunately the widely-grown variety, milo, has proved immune from all the smuts of sorghum" and he was therefore tempted to verify the statement. Accordingly the American authorities were requested to supply small quantities of seed of different kinds of milo sorghum. The result was complied with by supplying him with the seed of only one variety, Dwarf Milo[3] which was tried at the Agricultural College farm at Poona, India.

Before sowing, the seed was infected with the fresh spores of grain smut (*Sphacelotheca sorghi*) and loose smut (*Sphacelotheca cruenti*) [4]. Infection of the seed was effected by sprinkling the spores on the seed. The seed was sown June 19, 1920, and produced 635 heads of grain. Of these, 3 heads were affected by grain smut and 50 heads by loose smut; or 0.47 and 7.8 per cent respectively. It may therefore be said that Dwarf Milo sorghum is resistant but not immune to the grain smut, but is decidedly susceptible to the loose smut.—G. S. KULKARNI.

Personals.—Miss E. M. Wakefield, F. L. S., mycologist at the Kew Botanical Gardens, has finished a number of months' work in the British West Indies. On her way home she is visiting botanical institutions at New York; Washington, D. C.; Columbus, Ohio; St. Louis, Mo.; Toronto; Ithaca, N. Y.; and Boston. While in Washington she was tendered a dinner by the women mycologists and pathologists of the U. S. Department of Agriculture.

[1] Kulkarni, G. S. Smut of jowar (sorghum) in the Bombay Presidency. Agri. Research Inst. Pusa, Bull. 78, *p* 4. 1918.

[2] Potter, A. A. Head smut of sorghum and maize. Jour. Agric. Res. **2**: 368. 1914.

[3] Thanks are due to Dr. David Fairchild and G. P. Van Eseltine.

[4] Four kinds of smut occur on sorghum: Grain smut, loose smut, whole head smut, and long smut (*Polysporium filiferum*). Of these the first two are important.

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